

enzyme-based assay work in combination, and even reproduce precise results at an average 19.4% relative percent difference (RPD). Second, because OP/C causes harmful health effects at high doses, we investigated pesticide concentration of farmers' market tomatoes compared to supermarket tomatoes. Our results suggested OP/C in three samples: 2 out of 10 farmers' market tomato samples and 1 out of 5 supermarket tomato samples. The detection of OP/C surprised us, though, because locations claimed organic production. One explanation of this could be that sources other than the farmers contaminated the samples.

Medical Science

$\alpha 7$ nAChR NEGATIVE ALLOSTERISM: A PROMISING APPROACH FOR COGNITIVE DISORDERS. O. I. Alwassil¹, G. Abdrakhmanova², and M. Dukat¹, ¹Department of Medicinal Chemistry and ²Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Progression of Alzheimer's disease (AD) is associated with an increase of β -amyloid peptide 1-42 ($A\beta_{1-42}$) neurotoxic interactions with $\alpha 7$ neuronal nicotinic acetylcholine receptors (nAChRs) leading to tau protein hyperphosphorylation and neuron deficit. Through their neuroprotective abilities against toxic $\alpha 7$ nAChR- $A\beta_{1-42}$ interactions, $\alpha 7$ nAChR ligands might represent promising targets for AD symptomatic therapy. We have identified *meta*-chlorophenylguanidine (*m*CPG; $IC_{50} = 8.0 \mu M$) as the first small-molecule negative allosteric modulator (NAM) at $\alpha 7$ nAChRs. *m*CPG might serve as a lead in developing structure-activity relationships for NAMs at $\alpha 7$ nAChRs. Several analogs of *m*CPG were synthesized and evaluated in whole-cell patch-clamp assay. Introduction of a methyl group on the aniline nitrogen atom of *m*CPG resulted in a more potent $\alpha 7$ NAM ($IC_{50} = 1.3 \mu M$) than *m*CPG. Since the exact interaction site(s) and mechanism by which the $\alpha 7$ nAChR NAMs work has not been yet fully described, we developed 3D models of the extracellular domain (ECD) of human MBOL97\fr"Symbol"s107 nAChRs. Modeling studies resulted in the identification of two out of five binding sites in the ECD that are supported by empirical data. The different docking solutions are consistent with functional data. *Supported in part by the Virginia Center on Aging (Award No. 12-2).*

THE INTERACTION BETWEEN WIN55,212-2 AND RADIATION ON INHIBITING THE GROWTH OF BREAST CANCER CELLS. S. M. Emery¹, E. T. Sumner, Q. Tao, A. H. Lichtman & D. A. Gewirtz¹, ¹Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. Win55,212-2 (WIN2) is classified as a full agonist for cannabinoid receptors CB1 and CB2, as well as an agonist for known off targets of the endocannabinoid system including TRPV1 and PPAR α - γ . Recent reports have shown that WIN2 has anti-proliferative effects on cancer, but no studies have been performed to evaluate potential interactions between WIN2 and ionizing radiation (IR) used in cancer treatment. We have shown that WIN2 has the capacity to significantly enhance the anti-proliferative effects of IR in the MCF-7 breast tumor cell line. This effect has been reproduced in MDA-MB-231 and 4T1

breast tumor cells and in the syngeneic tumor model of 4T1 cells in Balb/c mice; of importance for selectivity, this combination effect does not transfer to the MCF-10a model of normal breast epithelial cells. Interestingly, in MCF-7 cells this enhancement of sensitivity is not observed for other drugs of the cannabinoid class, including CP 55,940, Methanandamide, Cannabidiol, Nabilone and Δ^9 -Tetrahydocannabinol. RT-PCR demonstrates that in MCF-7 cells neither CB1 nor CB2 are expressed and selective antagonists for each receptor failed to antagonize WIN2's actions. Although both TRPV1 and PPAR γ are expressed in MCF-7 cells, pharmacological studies using Capsazepine (TRPV1 antagonist), Capsaicin (TRPV1 agonist), GW9662 (PPAR γ antagonist), and Pioglitazone (PPAR γ agonist) demonstrate that neither TRPV1 nor PPAR γ are likely mediating the radiosensitizing effects of WIN2. Supported by a predoctoral training grant from the DOD to Sean Emery.

INVOLVEMENT OF CCR5 IN MORPHINE AND TAT-MEDIATED NEURODEGENERATION. E.M. Podhaizer¹, Y. Zhang², P.E. Knapp^{1,3}, K.F. Hauser¹. Depts. of ¹Pharmacol. & Toxicol., ²Med. Chem., ³Anat. & Neurobiol., Virginia Commonwealth Univ., Richmond, VA. Studies have shown that dual morphine & Tat treatment produce synergistic neurotoxicity in neuron-glia co-cultures. We chose to examine CCR5's role in this effect due to its interactions with the μ -opioid receptor, and its ligand's involvement in glial activation. We hypothesized that blockade/deletion of CCR5 would prevent morphine and Tat interactive neurodegeneration. A CCR5 antagonist, maraviroc (MVC), was used first to assess CCR5's role in morphine & Tat toxicity. While MVC had no effect on morphine or Tat toxicity alone, it blocked the enhanced effect of the combined treatment. Use of CCR5 knockout (KO) glia with wild-type (WT) neurons showed a delay in the onset of neurotoxicity in morphine, Tat, or dual-treated cells and the overall toxicity of the combined treatment was reduced. When WT glia and CCR5 KO neurons were used, morphine & Tat toxicity was significantly reduced, while morphine toxicity was enhanced. However, deletion of CCR5 in both neurons and glia showed significant toxicity under basal conditions and suggests that CCR5, per se, is neuroprotective. To determine if the glial component of the CCR5 response could be due to beta-chemokine release, CCR5's endogenous ligands, MIP-1 α , MIP-1 β , and RANTES were applied to co-cultured cells. While the highest concentrations did show significant neurotoxicity, physiologically relevant levels did not produce an effect, suggesting that elevated levels of CCR5 agonists were not responsible for enhanced morphine & Tat toxicity and suggest an alternative mechanism. Funding sources: R01 DA019398, T32 DA007027, F31 DA033203.

AGOUTI-RELATED PROTEIN (AGRP) RNA EXPRESSION IN THE VISCERAL ADIPOSE TISSUE OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) PATIENTS. David Van Natta^{1,3}, Christopher Gerner^{1,3}, Ancha Baranova^{1,2,3}, J. Michael Estep^{1,3}, 1, Biology Department, College of Science, George Mason University 2. School of Systems Biology, College of Science, George Mason University 3. Beatty Liver and Obesity Research Project, Institute of Research, Inova Health System. AgRP is an appetite regulating peptide that may play roles in inflammation and calcium homeostasis. Recently it was demonstrated that hypothalamic AgRP expression is regulated by FFA. This pilot study measures AgRP expression in the VAT of morbidly

obese patients in the context of metabolic disease. Of the 21 patients analyzed to date, 15 (71%) were female, 6 (60%), and 11 (52%) had non-alcoholic steatohepatitis (NASH) while the other 10 had non-NASH NAFLD (47%). Importantly, Patients with a diagnosis of NASH expressed AgRP significantly less than those with a diagnosis of non-NASH NAFLD (FD=-3.95, P=0.01).

PHARMACOLOGY OF ALPHA5* NICOTINIC ACETYLCHOLINE RECEPTORS IN MEDIATING ETHANOL- RESPONSIVE BEHAVIORS. A. J. Dawson¹, M.F. Miles¹, M.I. Damaj¹. Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Nicotine and Alcohol (ethanol) are two of the most widely abused drugs in society. Because of the high co-morbidity of addiction to these drugs, it is thought that nicotinic acetylcholine receptors (nAChRs), the principle mediators of nicotine addiction, may also contribute to alcohol addiction. Recent human genetic evidence suggests that the *CHRNA5/A3/B4* gene coding region, which codes for $\alpha 5^*$ nAChRs (* denotes the presence of additional subunits), is associated with both nicotine and alcohol dependence. While behavioral evidence exists that support this finding in nicotine-responsive behaviors in animals, there are virtually no published studies that have investigated the effects of $\alpha 5^*$ nAChRs on ethanol-responsive behaviors *in vivo*. We sought to determine the effect of $\alpha 5$ nAChR gene removal on the effects of acute ethanol exposure and chronic drinking behavior using high-ethanol preferring C57BL/6J null mutant mice. These $\alpha 5$ Knockout (KO) mice were tested for changes in acute ethanol-responsive behaviors including loss of righting reflex, hypothermia, and anxiolysis, as well as for modulation of drinking behavior. Results revealed that while $\alpha 5$ KO mice showed a general enhancement in their response to ethanol's acute effects, there was no difference in their drinking behavior. Furthermore, because $\alpha 5^*$ nAChRs must necessarily co-assemble with $\alpha 4\beta 2^*$ or $\alpha 3\beta 4^*$ nAChRs, we tested for the involvement these nAChR subtypes in the previously mentioned measures using pharmacologically and genetically manipulated C57BL/6J mice. Preliminary results showed that antagonism of these receptors differentially modulated some acute ethanol responses. Taken together, these data suggest a real, albeit, complex role for $\alpha 5^*$ nAChR subtypes in mediating initial sensitivity to some of the effects of acute ethanol exposure.

THE DINUCLEAR PLATINUM AGENT, BBR3610-DACH, INDUCES G1/S AND G2/M CELL CYCLE ARREST THROUGH DIFFERENT ROUTES IN HUMAN COLORECTAL CANCER HCT116 CELLS. Vijay Menon¹, E. Peterson², L.F. Povirk¹, N. Farrell^{1,2}, ¹Dept. of Pharmacology and Toxicology, and ²Dept. of Chemistry, Virginia Commonwealth University, Richmond, VA. BBR3610-DACH is a long-chain, bifunctional dinuclear Pt (II) complex shown previously to be resistant to metabolic decomposition by sulfur-containing nucleophiles. Initial observations utilizing the comet assay indicated that both BBR3610 and BBR3610-DACH formed interstrand crosslinks. However, from our subsequent studies, it is seen that both drugs induced different cellular effects with BBR3610-DACH causing a significant G1/S and G2/M cell cycle arrest in HCT116 cells in a time dependent manner with depletion in the S phase cells. The G1/S arrest was accompanied by a stabilization of p53 between 24 to 48 h and a concomitant increase in the cyclin dependent kinase inhibitors, p21 and p27.

An early decrease in the cyclin A level suggested a G2/M arrest. Studies with synchronized cells showed that BBR3610-DACH specifically inhibited the progression of G1 arrested cells into the S phase. Also, utilizing isogenic p53 and p21 null cell lines, it was seen that both p53 and p21 determine the sensitivity of BBR3610-DACH induced growth arrest. BBR3610-DACH also induced an early apoptosis along with an increase in the levels of cleaved PARP-1. Together these findings suggest that BBR3610-DACH is a major cell cycle inhibitory DNA binding platinum agent that could be developed further as a major chemotherapeutic.

IDENTIFYING GENES THAT INFLUENCE ACUTE ETHANOL RESPONSE BEHAVIORS IN *Caenorhabditis elegans*. J.T. Alaimo, A.G. Davies, J.C. Bettinger, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Alcohol dependence is a complex disorder that is strongly influenced by genetic factors. An individual's initial response to acute ethanol is a strong indicator of future abuse, but little is known about how acute ethanol exposure leads to changes in neuronal activity and behavioral responses. Previous studies have shown that acute ethanol treatment induces changes in expression of many genes that are important in ethanol responsive behaviors. Using the model organism *Caenorhabditis elegans*, we plan to survey gene expression changes induced by ethanol and construct gene networks to identify candidate functional loci. We predict that transcriptional changes that occur during and subsequent to the acute ethanol response are critical for changes in behavior. However, we hypothesized that acute responsive genes may be needed to observe the behavior. We asked if transcription of acute response causing genes is required for the behavior acute functional tolerance (AFT). We tested this by inhibiting transcription during acute ethanol exposure using actinomycin D and observing the development of AFT in two strains, wild type N2 and a mutant strain that rapidly develops AFT due to the lack of a functional neuropeptide Y receptor (*npr-1*). Both N2 and *npr-1* mutant animals retained the ability to develop AFT when transcription was inhibited, suggesting that transcription of the acute response genes is not required for AFT. We hope that uncovering ethanol responsive genes and their networks by transcriptional profiling will uncover important genes involved in ethanol responses.

MITOCHONDRIAL DNA AS A QUANTITATIVE MARKER OF BROWN ADIPOSE ACTIVITY. K. Doyle², A. Neupane^{1,2}, R. Mehta^{1,2}, Z. Younossi^{1,2,3}, A. Birerdinc^{1,2}, & A. Baranova^{1,2}, ¹Betty and Guy Beatty Center for Integrated Research, Inova Health System, ²School of Systems Biology, GMU, and ³Center for Liver Diseases, Inova Fairfax Hospital. Obesity is one of the biggest health problems in the United States and is linked to a number of degenerative diseases. Recent data suggests that brown adipose activity may play a role in both adult body weight homeostasis and the regulatory mechanisms of obesity. Unfortunately, brown adipose tissue related genes can remain dormant under certain conditions making it difficult to use gene expression studies to determine if it is present. However, a large number of mitochondria are found in this cell type and so we are using both mitochondria specific DNA and genomic DNA primers to quantify the amount of each type of DNA using qPCR. This data will be used to obtain a ratio that indicates the total amount of brown adipose tissue in the samples of subjects with and without NASH.

GENE EXPRESSION PATTERN ANALYSIS IN GASTRIC TISSUE OF PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE. E. Stolworthy¹, R. Mehta^{1,2,3}, L. Wang^{1,2,3}, A. Baranova^{1,2,3}, Z. Younossi^{1,2,3}, & A. Birerdinc^{2,3}, ¹ Biology Department of Science, George Mason University, Fairfax Virginia 22030. ²School of Systems biology, College of Science, George Mason University, Fairfax, VA 22030. ³Betty and Guy Beatty Center for Integrated Research, Inova Health Systems. Over the past few decades, obesity is accompanied with increasing incidence of Non-alcoholic fatty liver disease (NAFLD). As a result, there is great interest in identifying which genes differentially expressed in NAFLD. Gene expression in gastric tissue was evaluated by qPCR. Normalized data was subjected to group comparison, correlation analysis and regression analysis. Group comparison between NASH and no NASH cohort showed CCL1 (1.23, p=0.05), CCR3 (3.45, p=0.04), CCR9 (2.97, p=0.04), CXCL12 (0.65, p=0.05), IL1RN (3.96, p=0.01), IL5 (3.14, p=0.05), IL8RA (15.05, p=0.04), IL8RB (3.14, p=0.05), and IL9 (9.49, p=0.01) to be significantly differentially expressed. Similarly CXCL14 (1.77, p=0.03), IL1F10 (2.25, p=0.04), and IL8RB (1.57, p=0.02) were differentially expressed between Steatosis and no Steatosis cohort. Group comparison between Mild Inflammation and Advanced Inflammation found that CCL4 (0.43, p=0.03), CXCL2 (0.35, p=0.02), CXCL6 (0.23, p=0.03), IFNA2 (0.26, p=0.02), ILF19 (0.25, p=0.02), IL1F8 (.025, p=0.02), and IL8 (0.21, p=0.02) to be differentially expressed. These inflammatory genes may be helpful in understanding the gastric - liver axis in the pathogenesis of Non-Alcoholic Fatty Liver Disease.

A NOVEL DIAGNOSTIC APPROACH FOR CHRONIC SYSTEMATIC DISEASE: DISTANCE MEASURE OF GENE EXPRESSION PROFILE. Lei Wang^{1,2}, Ganiraju Manyam³, Boris Veytsman¹ & Ancha Baranova^{1,2}, ¹School of System Biol., George Mason Univ., Fairfax, VA. ²Ctr. of Liver Diseases, Inova Fairfax Hosp., Falls Church, VA. ³Betty and Guy Beatty Ctr. for Integrated Research, Inova Health System, Falls Church, VA. ³Dept. of Bioinformatics & Comp. Biol., The UT MD Anderson Cancer Ctr., Houston, TX. Protein or RNA biomarkers are widely used for diagnosis. However, identification of novel biomarkers usually suffers from either low specificity or unsatisfactory reproducibility. Here we propose a novel approach that measures global distance between entire gene express profiles. We use psoriasis, as model disease to demonstrate the feasibility of our new concept. We analyzed microarray data sets and used Mahalanobis distance as an estimation of the degree of differentiation. The effectiveness of global signatures was compared with that of traditional specific signatures. In conclusion, the global distances of gene expression profiles can serve as reliable good classifiers as specific signatures, and therefore it is worth considering using them as diagnostic markers.

ETHANOL-INDUCED DEFECTS IN OLFACTORY NEURON CELL FATE DECISIONS IN CAENORHABDITIS ELEGANS. L.M. Kondo, R.C. Raabe, K.S. Kauv, M.H. Bolling, A.G. Davies, & J.C. Bettinger, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. Fetal alcohol syndrome (FAS) is the leading preventable cause of mental retardation, but the underlying molecular mechanisms are not well understood. We have taken a genetic approach to studying the effect of ethanol on a discreet cell fate decision occurring during embryogenesis in the nematode, *C. elegans*. AWC cells are a pair of olfactory

neurons that allow *C.elegans* to discriminate between volatile attractive odorants in chemotaxis and odorant discrimination assays. Early in development, AWC neurons make an activity dependent cell fate decision, and subsequently, particular groups of G protein-coupled receptors are asymmetrically expressed. A GFP tagged receptor STR-2 (STR-2::GFP) allows us to monitor cell fate decisions between AWC neurons. SLO-1, a voltage-gated potassium channel is expressed in these neurons, and activation of this channel can lead to both AWCs adopting the same cell fate. Previous studies from our lab have shown that SLO-1 is a major molecular target of ethanol and mediates ethanol sensitivity. We tested ethanol exposure during embryogenesis and hypothesize that the interaction between ethanol and the SLO-1 channel alters AWC cell fate. Furthermore, by altering the lipid composition of the cell membrane, we can render this cell fate decision resistant to the effects of ethanol.

SULFATIDE REGULATION OF PARANODAL FORMATION IN THE PERIPHERAL NERVOUS SYSTEM. H.F. Herman^{1,2}, E.L. Kwong¹, and J.L. Dupree^{1,2}, ¹Dept. of Anatomy and Neurobiology and ²Molecular Biology and Genetics, Virginia Commonwealth University, Richmond, VA 23298. Sulfatide is a galactolipid and a major lipid component of the myelin sheath. Its production is catalyzed by the enzyme cerebroside sulfotransferase (CST). To determine the functions of sulfatide, the gene encoding CST was genetically disrupted resulting in mice incapable of sulfatide synthesis. We have employed a combination of immunocytochemistry and confocal microscopic analysis of the CST null mice to determine the role of sulfatide in protein cluster onset and maintenance in the PNS by quantifying the number of protein clusters in WT and CST KO mice of ages ranging from 4 days to 10 months. Neuronal voltage-gated sodium channels were decreased in the CST KO at 4 days of age, while clusters of the glial protein neurofascin 155 were decreased in the CST KO at 7 days of age, though both of these deficits were righted by 15 days of age. Qualitative ultrastructural studies were also undertaken to analyze the stability of the nodal and paranodal regions at these ages. Together, our results indicate that sulfatide plays a role in the onset of protein clustering in the node and paranode as well as in the formation and maintenance of the node and particularly the paranode in young mice, as transverse bands are rarely observed in the CST KO mice.

“BUBBLES” AND “BATH SALTS” AND DOPAMINE TRANSPORTERS. R. Kolanos¹, K. N. Cameron², L. J. De Felice² & R. A. Glennon¹. ¹Department of Medicinal Chemistry and ²Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond VA 23298. In the last few years there has been a worldwide increase in the abuse of psychoactive *bath salts*. Recent analyses indicate that the active ingredients in many brands of *bath salt* products contain mephedrone (a. k. a. “bubbles”) and methylenedioxypyrovalerone (MDPV), sometimes in combination with other synthetic cathinones such as methylone, methedrone and flephedrone. Surprisingly, very little is known about the mechanism of action of these synthetic cathinone derivatives despite an increasing number of *bath salts*-related hospitalizations and deaths. The limited studies on mephedrone and MDPV, along with the structural resemblance to methcathinone, suggested that their behavioral effects might have a dopaminergic mechanism similar to dopamine-releasing agents. In an effort to better understand the effect of synthetic cathinones on the human dopamine transporter

(hDAT) we synthesized some of the most common ingredients of *bath salts* and recorded currents through hDAT-expressing *Xenopusleavis* oocytes using a two-electrode voltage clamp technique. Our studies showed that mephedrone is behaving like a dopamine releaser similar to methcathinone and methamphetamine. MDPV, though containing a methcathinone core, is acting like a potent hDAT blocker similar to cocaine. In fact, the combination of mephedrone and MDPV might simultaneously increase dopamine release and inhibit dopamine reuptake, leading to an unusually high synaptic concentration of the neurotransmitter DA and, hence, the high abuse potential of *bath salt* products. Supported in part by NIH DA033930.

SULFATIDE REGULATES MEMBRANE ASSOCIATION OF NFASC155 AND MAG DURING MYELINATION. A.R. Hackett, A.D. Pomier, N. Purdy, J.M. DeLoyht & J.L. Dupree. Dept. of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond VA 23298. Mice incapable of synthesizing the myelin lipid sulfatide form normal paranodes that deteriorate with age. Mice that lack either the neuronal paranodal proteins contactin or caspr or the myelin paranodal protein neurofascin155 (Nfasc155), the 3 proteins that comprise the junctional complex that tethers the myelin sheath to the axon, also exhibit unstable paranodes. However, since sulfatide does not cluster in the paranode, has no paranodal binding partner and since sulfatide null mice express the 3 paranodal junctional proteins, it is unclear how the lack of sulfatide results in paranode instability. Here, we test the hypothesis that sulfatide maintains paranode stability by regulating the distribution of Nfasc155 through the formation of lipid rafts. Consistent with our hypothesis, clusters of Nfasc155 are unstable in the absence of sulfatide. We also show that the membrane association of myelin-associated glycoprotein, another myelin protein that maintains myelin-axon adhesion, also requires sulfatide while the membrane associations of structural myelin proteins are sulfatide independent. These findings are significant as the levels and fatty acid composition of sulfatide are altered in both neuronal and myelin degenerative diseases indicating a potential loss of myelin-axonal adhesion in disorders not typically associated with demyelination.

Posters

OPIOID AND HIV-1 ASSOCIATED NEURODEGENERATION: IMPLICATIONS FOR P2X₄ RECEPTOR INVOLVEMENT. M.E. Sorrell¹, S. Zou², P.E. Knapp^{1,2}, and K.F. Hauser¹. ¹Dept. Pharm. & Tox., ²Dept. Anat. & Neurobiology., VCU, Richmond, VA. HIV-1-associated neurocognitive disorders (HAND) is seen in 50% of AIDS patients. Individuals who abuse opiates can have an increased incidence of HAND with more symptoms. To test HIV-1 and opioid-induced neurotoxicity are mediated by purinergic signaling, co-cultures of primary neurons and mixed glia were treated with combinations of Tat, morphine, and TNP-ATP, a P2X antagonist. Tat and morphine neurotoxicity was reversible by treatment with TNP-ATP. Tat + morphine decreased dendritic length, this was prevented by TNP-ATP treatment. Tat and morphine increased intracellular Ca²⁺ levels, this was preventable with TNP-ATP present. Antagonists against the P2X₁, P2X₃, and P2X₇ receptor subtypes were screened. Findings showed that these subtypes were not involved in Tat + morphine neurotoxicity supporting that P2X₄ receptors are involved. Human P2X microarray data from HIV

infected and non-infected individuals [courtesy NNTC] suggest that P2X₄ is altering neuroAIDS and the P2X₄ receptor may be a novel therapeutic target for HAND. *Support: NIH DA018633, DA028741, DA007027, and the NNTC.*

ASSOCIATIONS BETWEEN SLEEP DIFFICULTIES AND WELL BEING IN BREAST CANCER SURVIVORS. Anthony Loria^{1,3}, Patrice Winter^{1,3}, Katherine Doyle^{1,3}, Yang Wang^{2,3}, Lynn Gerber^{1,2}, Ancha Baranova^{1,3} & Zobair Younossi¹, 1. Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, United States 2. Center for the Study of Chronic Illness and Disability, College of Health and Human Services, George Mason University, Fairfax, VA, United States 3. George Mason University, Fairfax, VA, United States. Sleep difficulties are often documented in cancer patients following treatment. The aim of this study was explore the relationships between sleep difficulties and well being. Self report of well being (FACIT-F) and blood samples were collected from women with breast cancer who were more than 3 months past primary treatment. Measures of sleep difficulty were analyzed with respect to pro-inflammatory cytokines, C reactive protein and subscales of the FACIT-F and ascribed for significance (Mann-Whitney U tests). Forty-seven women [57 ± years] of whom 67 % reported sleep difficulties were binarily examined by 25th and 75th percentile with respect to subscales. Social, Emotional and Functional well being were all significantly different between those with and without sleep difficulties ($p \leq .032$, $Z \geq -2.143$). In the fatigue subscale, TGF-β1 (-1.899, .058) and C reactive protein (-1.833, .067) were approaching significance. Sleeping problems may be influenced by multiple domains of function and biological homeostasis in breast cancer survivors.

SUMMER ANATOMY INTERNSHIP FOR OMS-I: AN OPPORTUNITY FOR AUGMENTED TRAINING. R.P. Wyeth, K. D'Amato, I. Danelisen, J. Anstrom. VCOM Anatomy Department and the Plastination Laboratory, Via College of Osteopathic Medicine, Virginia Campus, Blacksburg VA 24060. During the 2011 summer recess an internship was offered to selected first year students based on both academic achievements and anatomical interests. The internship had two purposes: 1) provide students an opportunity to increase their anatomical knowledge in an area of special interest, and 2) develop expertly dissected specimens that could be subsequently plastinated and used as anatomy teaching aids in future years. Sixteen students, working individually or in small groups, chose a dissection project and worked under supervision of the authors. An anonymous survey was administered at the conclusion of the internship with 15 of 16 participants responding. Six five-level Likert items were included in the questionnaire, which was distributed online. Compilation of the results indicates that: self assessment of the student's anatomical knowledge increased by 87% when compared to perceived knowledge prior to the internship ($p < 0.05$); available facilities were adequate (73%); responsiveness of the faculty was viewed favorably (94%); available time was adequate (67%); and a majority of participants would recommend such an internship program to future students (86%). Thus, the summer anatomy internship is a valuable new adjunct to anatomy teaching that demonstrates a beneficial impact on participating students' knowledge while providing the medical community with well-prepared prosected specimens for plastination. *Supported by Seed Money from the VCOM Foundation as a part of the Plastination Project.*

ANOMALY OF THE CIRCUMFLEX CORONARY ARTERY IN A CONTINUOUS SERIES OF 338 VIRGINIA CADAVERS. R.P. Wyeth, A. Santo, K. Lester, S. Wachob, A. Petty, J. Bookbinder. Via College of Osteopathic Medicine, Virginia Campus, Blacksburg VA 24060. Canonically, the left main coronary artery originates in the posterolateral left sinus of Valsalva from a single ostium while the RCA begins in the right sinus of Valsalva arising from a single ostium. The left main coronary artery then bifurcates into the left anterior descending (LAD) and circumflex arteries (LCX). The LAD courses along the anterior interventricular sulcus giving rise to a variable number of branches. The LCX typically departs the left main coronary artery turning sharply laterally and posterior terminating in obtuse marginal branch(es). Most commonly the LCX terminates immediately distal to its obtuse marginal branch(es). Infrequently the LCX continues towards the crux cordis reaching the posterior interventricular sulcus producing the posterior interventricular branch (PDA). More commonly the RCA gives rise to the PDA. These hearts are considered “right dominate”. If the LCX terminates as the PDA the heart is termed “left dominate.” In this current study, the LCX transverses the poster crux cordis without giving rise to the PDA. That is, arising from the opposite aortic ostium, the right ostium, following a retroaortic course. We report here the occurrence of a LCX arising from its own ostium within the right sinus of Valsalva and coursing retroaortic within the left atrioventricular sulcus to supply the diaphragmatic aspect of the heart terminating in the marginal branches. This is the first LCX variation in a continuous series of 338 human hearts (an incidence of 0.3%) studied in conjunction with the Human Anatomy Course at the Via College of Osteopathic Medicine.

GENDER AND TIME DIFFERENCES OF NON-INVASIVE BLOOD PRESSURES IN THE SIEGEL LARGE AND SMALL LINE OF CHICKEN—INFERENCE FOR A GALLIFORM MODEL OF THE METABOLIC SYNDROME. N. Sheth¹, R. Clark¹, L. Solis Lopez¹, P.B. Siegel², R.P. Wyeth¹. ¹Via College of Osteopathic Medicine, Virginia Campus. ²Dept. of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg VA 24060. Cardiovascular disease is the leading cause of adult death. Systemic blood pressure (NIBP) is a function of cardiac power, circulating volume and vascular tonus. The current study compares NIBP to determine if weight differences and gender differences affect hypertension. A total of 16, age matched, chickens were used: 4 high weight females (HWF), 4 high weight males (HWM), 4 low weight females (LWF) and 4 low weight males (LWM). Pressures were obtained from the brachial artery. The total means of systolic, diastolic, and calculated mean of each day for each line were then averaged for class of bird and gender for type NIBP perimeter. The body mass of chickens in this study demonstrated significant differences in weight HWM and LWM and between LWF ($p = 0.001$). This study showed gender differences between NIBP, the male blood pressures were significantly higher in diastolic ($p = 0.011$), systolic ($p = 0.002$) and calculated mean ($p = 0.004$). As time increased, NIBP increased in only LWM while NIBP decreased in HWM, LWF, and HWM. The inconsistencies of NIBP on Day 491 are explained by change to a female handler. The current study demonstrates that significant gender related differences in NIBP. The study also illustrates that in these lines, blood pressure decreased with time. Further research is needed to ascertain if (handler sex vs. chicken sex) gender differences plays a role in NIBP monitoring.

GENDER AND BODY MASS DIFFERENCES IN SEROTONERGIC FEMORAL ARTERIAL RESPONSES IN A GALLIFORM MODEL OF THE METABOLIC SYNDROME. N. Sheth¹, R. Clark¹, L. Solis Lopez¹, P.B. Siegel², R.P. Wyeth¹. ¹Via College of Osteopathic Medicine, Virginia Campus, Blacksburg. ²Dept. of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg VA 24060. The metabolic syndrome (MES) predisposes to atherosclerosis inducing platelet activation and degranulation releasing several factors including 5-HT. 5-HT induces vessel vasoconstriction and platelet aggregation capable of causing ischemia and infarction. The current study hypothesizes that there is no difference in 5-HT vasomotor activity with respect to body mass and gender in a galliform model of MES. This was tested in ischiatic arteries. Samples were constricted with 5-HT. Analysis of the aorta showed neointimal hyperplasia and atheroma consistent with disease process in humans. 5-HT stimulation produced dose response curves similar to those seen in human arteries. Efficacy, determined by maximum tension developed, was different in high weight females (HWF). While potency, as 50% of the concentration required to reach maximum contraction approached significance in HWF vs. low weight (LWF), and was significant in LWF vs HWF ($p < 0.003$). Thus, a significant difference was noted in 5-HT potency between weight lines. The current study illustrates that, when comparing these high weight and low weight chickens: 1) 5-HT significantly modulates maximal contraction in HWF. 2) There is significant difference in potency (EC_{50}) between HWF and LWF, and HW male (M) and LWM. These initial findings suggest that obesity and gender differentially effect 5-HT activity and are significant effectors of 5-HT induced vasospasm, sensitive to both gender and body mass in this MES model.

REAL-TIME PCR BASED APPROACHES TO THE QUANTIFICATION OF BROWN ADIPOCYTE ACTIVITY WITHIN WHITE ADIPOSE DEPOTS. A.S. Neupane^{1,2}, A. Baranova^{1,2}, A. Bircerdinc^{1,2} & R.Mehta^{1,2,3}, ¹Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, ²Center for the Study of Genomics in Liver Diseases, Molecular and Microbiology Department, George Mason University, Fairfax, VA and ³Center for Liver Diseases, Inova Fairfax Hospital. The pandemic of obesity has been fueled by the easy access to high-calorie diet and an increasingly disengaged lifestyle brought upon by technological advancement. This not only poses a severe threat to the health of individuals, but also extends the adversity to the already overburdened global public health. Mammals, equipped with thermogenic brown adipose tissue (BAT), have evolved a unique and efficient biological mechanism to dissipate large amounts of stored energy as heat. Contrary to the energy storage function of white adipose tissue, BAT is a mitochondria rich tissue which, with the help of UCP1 protein, uncouples energy production and thus functions as an energy dissipating organ. Hence, it is highly plausible that BAT upon activation may contribute to the process of energy homeostasis. Until recently, the presence of active BAT in adult humans was highly debated. The possibility of reduced amounts or dysfunctional BAT as the underlying cause of obesity is a tantalizing and new approach in addressing the obesity epidemic. In this study, we attempt to detect the presence of BAT using novel approach. The ratio of mitochondrial DNA over nuclear DNA will be used as an indicator of the presence of mitochondria rich BAT in the visceral adipose depot of morbidly obese patients with and without NAFLD.

RT-PCR PROFILING OF MITOCHONDRIAL AND GENOMIC DNA IN VISCERAL ADIPOSE TISSUE OF NASH AND NON-NASH PATIENTS. L. Singh^{1,2}, K. Doyle², R. Mehta², A. Birerdinc^{1,2}, A. Baranova^{1,2} & Z. Younossi^{1,3}, ¹Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, ²Center for the Study of Genomics in Liver Diseases, School of Systems Biology, George Mason University, Fairfax, VA and ³Center for Liver Diseases, Inova Fairfax Hospital. The increased prevalence of obesity has placed great emphasis on brown adipose tissue (BAT) and its role in energy homeostasis and body fat regulation. BAT characteristically has high levels of mitochondria and is distinguished by the presence of uncoupling protein 1 (UCP1) in its inner mitochondrial membrane. Data indicates an inverse relationship between body mass index (BMI) of an adult and BAT activity. In order to quantify the presence of BAT in this study, mitochondrial DNA and genomic DNA were extracted from the visceral adipose tissue of 150 morbidly obese patients with and without NASH. The resulting ratio of genomic and mitochondrial DNA served as an indicator of mitochondrial levels in NASH and non-NASH patients regardless of its activation. Custom primers were designed and specificity verified by NCBI BLAST. QIAamp DNA mini kit was used to extract the total DNA. qPCR was done to target the genomic and mitochondrial DNA. Melt curves were analyzed for product specificity. The threshold cycle (C_t) from the amplification plot was then used to calculate the mitochondrial DNA to genomic DNA expression ratio.

ASSESSMENT OF VITAMIN D LEVELS AND PARATHYROID HORMONE (PTH) LEVELS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD). Mariam Hashemi¹, Massih Abawi^{1,2}, Rohini Mehta^{1,2}, Lei Wang¹, Zobair M. Younossi², Ancha Baranova^{1,2} & Aybike Birerdinc^{1,2}, ¹Center for the Study of Genomics in Liver Diseases, College of Science, George Mason University, Fairfax, VA ²Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA. The liver has an important function in processing Vitamin D, a fat-soluble vitamin which can be obtained from sun exposure and is naturally present in foods and dietary supplement. However, patients with Nonalcoholic Fatty Liver Disease (NAFLD) are usually deficient in Vitamin D. Parathyroid hormone (PTH) increases the activity of 1- α -hydroxylase enzyme, which converts 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, the active form of Vitamin D in the kidneys. . The purpose of this study is to determine the correlation between the presence of Vitamin D and the presence of PTH, which regulates the body's calcium levels. Clinical samples and data were obtained from 39 morbidly obese patients during bariatric surgery. Vitamin D and PTH levels were determined in the serum using enzyme-linked immunosorbent assay (ELISA). Circulating levels of Vitamin D and PTH in the serum were inversely correlated with each other within the NASH cohort ($r = -0.29$, $p < 0.05$). However, no significant correlation was found between PTH and Vitamin D levels when comparing the NASH and non-NASH cohorts ($r = -0.26$, $p > 0.05$). In conclusion, Vitamin D and PTH levels are negatively correlated with each other significantly only in the NASH cohort.

THE ROLE OF SULFATIDE IN MEDIATING OLIGODENDROCYTE MORPHOLOGY THROUGH TAU EXPRESSION. J.M. DeLoyht & J.L. Dupree, Dept. of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond

VA 23298. During development, oligodendrocyte (OL) lineage cells progress from simple bipolar cells to complex branched cells. The transition from this simple to the complex morphology temporally corresponds with the appearance of the OL sphingolipid sulfatide. Sulfatide has been identified as a potential regulator of OL differentiation and morphology. Our laboratory has reported that mice incapable of synthesizing sulfatide maintain OLs that exhibit a less complex morphology by extending fewer myelin forming processes than wild type (WT) mice. Since cellular morphology is regulated by cytoskeletal elements, we investigated the distribution and phosphorylation state of the microtubule associated protein tau. Phosphorylated tau was not observed in either OLs of 15 day old WT or sulfatide null mice. Surprisingly, accumulations of phosphorylated tau were observed in OLs of both WT and null OLs at 1 month of age. By 7 months of age, the prevalence of tau clusters was maintained in the WT cells but increased in the sulfatide null cells. Based on our data we propose that phosphorylated tau plays a role in normal OL development and myelination; however it remains to be determined how and why sulfatide depletion results in an increased accumulation of phosphorylated tau in OLs.

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DRAGONFLY PERCH SELECTION RELATED TO PERCH HEIGHT AND LOCATION. Jessica Beard & Deborah Waller, Dept. of Biological Sciences, Old Dominion Univ., Norfolk, VA 23529. A community of dragonflies (Odonata) was studied in July and August, 2011, at Hoffer Creek Wildlife Preserve, Portsmouth, VA. Male dragonflies patrol territories to secure food and mates and rest on perches. Two experiments were conducted with bamboo poles to study perch selection by adult males in relation to perch height and location, respectively. Four out of eight species present competed for the experimental perches (*Pachydiplax longipennis*, *Brachymesia gravia*, *Celithemis eponia*, and *Libellula needhami*). In the first experiment, two species used short perches (30cm) most frequently and two species used tall perches (90cm) over short perches. Perch height selection was not related to dragonfly size. Pole tops were preferred perching sites for all species but *L. needhami* frequently perched mid-pole. *Brachymesia gravia* was the dominant perching species in the beginning of the season and the least common species at the end of the season. In the second experiment, perches farther from shore (2m) were selected more frequently than those closer to shore (0.5m), regardless of perch height. Overall, species differences in perch height and seasonal use of perches could have implications in niche partitioning and competition among these species.

DEMOGRAPHY OF THE MEADOW VOLE (*MICROTUS PENNSYLVANICUS*) IN SOUTHEASTERN VIRGINIA. Jana F. Eggleston & Robert K. Rose, Dept. of Biological Sciences, Old Dominion Univ., Norfolk, VA 23529. We conducted a mark-capture-release (MCR) monitoring program of the meadow vole on the Su Tract from 2002 through 2005 and began one on the Stephens Tract in 2005. These sites are a part of the Nature Conservancy Stewardship of lands and were acquired via the Virginia Wetland Restoration Trust Fund as mitigation sites on the Northwest River drainage basin in Chesapeake, Virginia. On both tracts we established an 8 x 8 research grid, at 12.5m intervals, and with two modified Fitch traps per station. We